

Doctoral thesis at the University of Lund

The Role of Sex Chromosomes and Sex Chromosomal Genes in Vertebrate Sex Determination

by

Ulf H. Wiberg



University of Lund

SWEDEN

Lund 1985



Digitized by the Internet Archive
in 2026

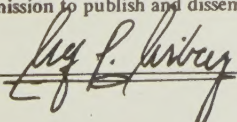
https://archive.org/details/IA41546224_0028

Organization LUND UNIVERSITY		Document name DOCTORAL DISSERTATION			
Institute of Genetics Sölvegatan 29 S-223 62 Lund, Sweden		Date of issue April 12, 1985			
		CODEN: LUNBDS/NBGE-1009/001-110/(1985)			
Author(s) Ulf H. Wiberg		Sponsoring organization			
Title and subtitle The role of sex chromosomes and sex chromosomal genes in vertebrate sex determination.					
Abstract This dissertation focuses on problems related to sex chromosomes, sex chromosomal genes and sex determination. As a representative of the lower vertebrates, the European eel was studied, and of the higher vertebrates three mammalian species; the mouse, the wood lemming and man. Histological, cytological, immunological and biochemical methods were used. The eel was shown to lack heteromorphic sex chromosomes. Female eels, however, express H-Y antigen (H-Y) (the proposed gonadal determiner of the heterogametic sex), suggesting heterogamety in the female of this species. A hypothesis on metagametic sex determination in the eel was put forward. Wood lemmings of the XY, XX, X*Y, X*X, X*O and XO sex chromosome types, and one X*XY male and one female, were studied for cellular expression of STS (steroid sulphatase), for which the gene in man has been shown to be X-linked and to escape X-inactivation. In the wood lemming a gene dosage effect was also found, corresponding to the number of X chromosomes, which indicates X-linkage and non-inactivation of the STS gene. X-inactivation (late X-replication) studies in the X*XY animals further revealed that in all cells of the male studied, the mutant X* was inactivated, in contrast to the female where in all cells the wild-type X was inactivated, implying that the type of active X determines sexual phenotype in animals with this sex chromosome constitution. (cont. overleaf)					
Key words mammals, man, wood lemming, mouse, fish, eel, sex chromosomes, sex chromosomal genes, sex determination, heterogamety, metagamy, STS, H-Y, X-inactivation, sexual phenotype, transplantation, serology					
Classification system and/or index terms (if any)					
Supplementary bibliographical information			Language English		
ISSN and key title			ISBN		
Recipient's notes	Number of pages 110		Price		
	Security classification				

Distribution by (name and address) Ulf H. Wiberg, University of Lund, Institute of Genetics, Sölvegatan 29, S-223 62 Lund, Sweden.

I, the undersigned, being the copyright owner of the abstract of the above-mentioned dissertation, hereby grant to all reference sources permission to publish and disseminate the abstract of the above-mentioned dissertation.

Signature



Date February 28, 1985

Abstract, cont.

H-Y antigen was also investigated in man, the mouse and the wood lemming. Serological H-Y typing was carried out in mouse and lemming, and transplantation typing in lemming and man. Taken together, the H-Y studies revealed that female XO mice are H-Y negative ($H-Y^-$) while XO humans and XO lemmings are $H-Y^+$, and that X^*Y and X^*O lemmings are $H-Y^+$. The H-Y data of the lemming (combined with H-Y data of man, obtained by others) led to the proposal of a model for the genetics of mammalian sex determination, involving genes on the X-chromosome, a Y-linked controlling gene and non-Y-linked H-Y structural genes.

In addition, the H-Y and STS data allowed for the following conclusions:

- i) The X of lemming and man carries a gene controlling H-Y expression and a gene for the H-Y antigen receptor (both genes lost by the wood lemming X^*); the former gene escaping X-inactivation.
 - ii) The difference of H-Y expression in mouse on the one hand and man and lemming on the other may be caused by corresponding differences in X-inactivation pattern; one of the Xes in the female mouse being completely inactivated while in man and lemming a small segment, containing the X-linked H-Y controlling gene, remains active.
 - iii) The H-Ys as defined by serology and transplantation are most likely identical.
 - iv) The H-Y structural gene(s) of man and lemming is not Y-linked.
- Methodological problems of H-Y serology were critically discussed and it could be summarized that:
- v) Only with thorough knowledge of the different parameters influencing H-Y serological tests is it possible to perform and interpret these tests correctly.
 - vi) Mammalian XX females lack the H-Y antigen (i.e. they are $H-Y^-$).
 - vii) No evidence for quantitative differences of H-Y antigen in XY males exists.

THE ROLE OF SEX CHROMOSOMES AND SEX CHROMOSOMAL GENES
IN VERTEBRATE SEX DETERMINATION

by

ULF H. WIBERG

Fil. kand. (Mlm)

Academic thesis for the degree of Doctor of Philosophy
to be publicly defended in the lecture hall of
the Institute of Genetics, Lund, on
Friday, April 12, 1985, at 10 a.m.

The thesis will be defended in English.

Doctoral thesis at the University of Lund 1985

© Ulf H. Wiberg

Printed in the Federal Republic of Germany by
Druckerei Johannes Krause, D-7801 Umkirch, 1985

Dedicated to my wife and my children,
and to the memory of my parents

This dissertation is based on the following papers which will be referred to by the Roman numerals I - IX.

- I. WIBERG, U.H. (1983). Sex determination in the European eel (Anguilla anguilla, L.) - A hypothesis based on cytogenetic results, correlated with the findings of skewed sex ratios in eel culture ponds. Cytogenet. Cell Genet. 36, 589-598
- II. ROPERS, H.H. and WIBERG, U. (1982). Evidence for X-linkage and non-inactivation of steroid sulphatase locus in wood lemming. Nature 296, 766-767
- III. SCHEMPP, W., WIBERG, U. and FREDGA, K. (1985). Correlation between sexual phenotype and X-inactivation pattern in the X*XY wood lemming. Cytogenet. Cell Genet. 39, 30-34
- IV. WIBERG, U. (1982). Serological Cross-Reactivity to Rat Anti H-Y Antiserum in the Female European Eel (Anguilla anguilla). Differentiation 21, 206-208
- V. WIBERG, U., MAYEROVÁ, A., MÜLLER, U., FREDGA, K. and WOLF, U. (1982). X-linked Genes of the H-Y antigen System in the Wood Lemming (Myopus schisticolor). Hum. Genet. 60, 163-166
- VI. WIBERG, U.H. and GÜNTHER, E. (1985). Female wood lemmings with the mutant X*-chromosome carry the H-Y transplantation antigen. Immunogenetics 21, 91-96

VII. WIBERG, U.H. (1985). H-Y transplantation antigen in human XO females. Hum. Genet. 69, 15-18

VIII. MAYEROVÁ, A., MÜLLER, U., WIBERG, U., WOLF, U. and FRACCARO, M. (1984). Variability in the amount of serologically detectable H-Y antigen. Hum. Genet. 66, 110-112

IX. WIBERG, U.H. and MAYEROVA, A. Serologically H-Y antigen negative XO mice (submitted to Journal of Immunogenetics)

THE ROLE OF SEX CHROMOSOMES AND SEX CHROMOSOMAL GENES
IN VERTEBRATE SEX DETERMINATION

ABSTRACT

Wiberg, U.H. (1985). The role of sex chromosomes and sex chromosomal genes in vertebrate sex determination., pp. 1-110. Doctoral thesis, University of Lund, Lund, 1985.

This dissertation focuses on problems related to sex chromosomes, sex chromosomal genes and sex determination. As a representative of the lower vertebrates, the European eel was studied, and of the higher vertebrates three mammalian species; the mouse, the wood lemming and man. Histological, cytological, immunological and biochemical methods were used.

The eel was shown to lack heteromorphic sex chromosomes. Female eels, however express H-Y antigen (H-Y) (the proposed gonadal determiner of the heterogametic sex), suggesting heterogamety in the female of this species. A hypothesis on metagametic sex determination in the eel was put forward.

Wood lemmings of the XY, XX, X*Y, X*X, X*O and XO sex chromosome types, and one X*XY male and one female, were studied for cellular expression of STS (steroid sulphotase), for which the gene in man has been shown to be X-linked and to escape X-inactivation. In the wood lemming a gene dosage effect was also found, corresponding to the number of X chromosomes, which indicates X-linkage and non-inactivation of the STS gene.

X-inactivation (late X-replication) studies in the X*XY animals further revealed that in all cells of the male studied, the mutant X* was inactivated, in contrast to the female where in all cells the wild-type X was inactivated, implying that the type of active X determines sexual phenotype in animals with this sex chromosome constitution

(cont. overleaf)

Abstract, cont.

H-Y antigen was also investigated in man, the mouse and the wood lemming. Serological H-Y typing was carried out in mouse and lemming, and transplantation typing in lemming and man. Taken together, the H-Y studies revealed that female XO mice are H-Y negative ($H-Y^-$) while XO humans and XO lemmings are $H-Y^+$, and that X^*Y and X^*O lemmings are $H-Y^+$. The H-Y data of the lemming (combined with H-Y data of man, obtained by others) led to the proposal of a model for the genetics of mammalian sex determination, involving genes on the X-chromosome, a Y-linked controlling gene and non-Y-linked H-Y structural genes.

In addition, the H-Y and STS data allowed for the following conclusions:

- i) The X of lemming and man carries a gene controlling H-Y expression and a gene for the H-Y antigen receptor (both genes lost by the wood lemming X^*); the former gene escaping X-inactivation.
- ii) The difference of H-Y expression in mouse on the one hand and man and lemming on the other may be caused by corresponding differences in X-inactivation pattern; one of the Xes in the female mouse being completely inactivated while in man and lemming a small segment, containing the X-linked H-Y controlling gene, remains active.
- iii) The H-Ys as defined by serology and transplantation are most likely identical.
- iv) The H-Y structural gene(s) of man and lemming is not Y-linked.

Methodological problems of H-Y serology were critically discussed and it could be summarized that:

- v) Only with thorough knowledge of the different parameters influencing H-Y serological tests is it possible to perform and interpret these tests correctly.
- vi) Mammalian XX females lack the H-Y antigen (i.e. are $H-Y^-$).
- vii) No evidence for quantitative differences of H-Y antigen in XY males exists.

PROLOGUE

"[The] evidence from triploids [fruit flies] gives no specific information as to the occurrence of genes for sex determination. If we think of the chromosomes only in terms of genes, it follows that genes are involved, but the evidence does not show what they are like. Even if genes are involved, we cannot state whether there is one gene in the X that stands for femaleness, or hundreds of such genes. Similarly for the ordinary chromosomes - the evidence does not tell us whether the genes for maleness, if there be such, are on all the chromosomes or on only one pair."

T.H. Morgan (1926)

The present dissertation deals with the genetics of sex determination. Like Thomas Hunt Morgan (Allen 1978), my objective has been to use genetics as a tool, the purpose being a better understanding of development.

The duality of sex and the possibility of tracing morphological events of sexual development backwards in ontogenesis makes sex determination a unique model system for the understanding of developmental events.

I hope that my contribution, in form of the papers summarized in this dissertation, has brought us a little further towards an understanding of the genetics of sex determination.

SEX CHROMOSOMES AND SEX DETERMINATION

(Paper I)

The problem of what determines sex has been debated since ancient times and has given rise to hundreds of hypotheses. Late in the last century Henking (1891), studying sperm development in the insect Pyrrhocoris apterus, observed that one chromosome ("peculiar chromatin element") always lagged behind the others in the meiotic division. It seemed to him a most unusual behaviour, and like a mathematician who puts aside an X quantity as an unknown to be solved later, Henking did not interpret this phenomenon further and called the laggard an X chromosome.

It was not until the beginning of the twentieth century that an answer, which had any relation to the underlying facts, was found. Cuénot (1899) wrote: "It is rather humiliating to state that as regards man and other mammals no advance has been made since the time of the predecessors of Aristotle, even though a considerable amount of work has been expended in trying to solve this problem [the problem of sex determination]; evidently the wrong approaches were chosen".

Some years later, McClung (1902) and Wilson (1905) identified the X chromosome as the one which determines the sex in insects. Wilson established the fact that the female contains two X chromosomes in every nucleus, and the male only one. Thus, the male had an odd diploid chromosome

number - one chromosome less than the female.

However, later it was found by different investigators that in many other animals the male does have the same diploid number as the female. Where the genetic mechanism of sex determination consists of a single pair of chromosomes, these are homologous throughout their length (homomorphic) in one sex (X-chromosomes) and non-homologous or only partly so (heteromorphic) in the other sex (X- and Y-chromosomes).

In 1961 it was shown by Ohno that in the fowl, Gallus domesticus, the female has one X chromosome and the male two; evidence for a Y-chromosome in the female was first obtained by Frédéric (1961) and later by Schmid (1962).

The XX sex is said to be homogametic, since it produces only one kind of gamete, while the XY sex is said to be heterogametic, because it produces two kinds of gametes in equal numbers. In the case of female heterogamety the X and Y chromosomes are often referred to as the Z and W respectively and, although not generally accepted, this terminology will be used here.

We can accordingly distinguish between an XX/XY type and a ZW/ZZ type of sex chromosome mechanism. In vertebrates both types exist; mammals and certain species of reptiles and amphibians have the XX/XY type, birds and certain other species of reptiles and amphibians have the ZW/ZZ type of sex chromosome mechanism. Among fishes, heteromorphic sex chromosomes are exceptions, if they occur at all (I), but heterogamety is found in fish (Ohno 1974).

Sex determination may be syngamic (i.e. sex is fixed at the time of gametic fusion) or metagamic (sex is established by environmental factors at some stage after fertilization or it remains in a labile state, subject to the influence of external factors). In placental mammals sex determination is always syngamic and it seems that in species with heteromorphic sex chromosomes the environment has no influence on sex determination, while in species lacking heteromorphic sex chromosomes metagamic sex determination has been found in some species and may be more frequent than is presently thought.

As just mentioned, it is doubtful whether heteromorphic sex chromosomes occur in fishes (Ohno 1974 and paper I). Still, there have been some claims of heteromorphic sex chromosomes in fishes, namely in the deep-sea fish Bathylagus wesethi (Chen and Ebeling 1966), in two other species of this genus and in some species of Fundulus (Chen and Ruddle 1970), in a taxonomically undescribed Mexican cyprinodont fish (Uyeno and Miller 1971), and in eels; e.g. the European eel (Anguilla anguilla) (see Table 1 in paper I). It should be noted that all these studies were carried out before the era of chromosome banding and that in some cases neither mitotic nor meiotic chromosomes of the "homogametic" sex were analyzed.

Since intraspecific chromosomal polymorphism can be quite extensive in certain fish species (Ohno 1967), the mere finding of a heteromorphic chromosome pair in individual fish does not necessarily indicate the presence of morphologically differentiated sex chromosomes.

In paper I, the claimed existence of sex chromosomes in the European eel was reinvestigated with chromosome banding techniques. With the use of chromosome C-banding and NOR-staining it could be demonstrated that a heteromorphic pair of chromosomes frequently occurred in cells of the specimens investigated but this heteromorphism was not related to sex. Instead, with the staining methods mentioned, it could be established that the heteromorphism was due to differences in the amount of constitutive heterochromatin (C-band) of the short arms of one chromosome pair. Furthermore, using the G-banding method, it was demonstrated that the largest and smallest metacentric chromosomes, claimed by other investigators to constitute a sex chromosome pair, in both sexes and a hermaphrodite, had their homologues. It was therefore concluded that in the European eel, no heteromorphic sex chromosomes exist. These results and conclusions were recently confirmed by Sola et al. (1984).

The European eel is a classical example of piscine intersexuality, and the most characteristic feature of sexual development in eels appears to be the long period during which the gonads remain undifferentiated. Although they finally seem to develop either as males or females, sex reversal has been reported even in mature eels (D'Ancona 1960).

The presence of heteromorphic sex chromosomes, as originally claimed, was thus surprising. The findings presented in (I), that the European eel lacks heteromorphic sex chromo-

somes, makes it easier to understand the intersexual character of this species. Since, in addition, there have been many reports that eels cultured in commercial ponds are overrepresented by males ($>90\%$), and since the temperature in such ponds, in order to promote growth rate, is usually much higher than in natural habitats of the species, a hypothesis on sex determination in the eel was put forward (I). It was suggested that high temperature favours male development. Important for this hypothesis was also the consideration of experimental evidence that temperature may, indeed, influence or determine sex in fish (Conover and Kynard 1981), sea turtles (Morreale et al. 1982) and crocodiles (Ferguson and Joanen 1982).

SEX CHROMOSOMAL GENES AND X CHROMOSOME INACTIVATION

(Papers II and III)

According to the discussion in the preceeding section, those genes which are located on the X, Y, Z and W chromosomes are sex chromosomal genes. These are not to be confused with sex determining genes which may or may not be located in the sex chromosomes and which will be treated separately later.

There is a large body of evidence that the mammalian X chromosome is evolutionarily conserved. Conservation of the original X implies that there should be extensive homology of the X-linked genes among placental mammals. Anatomical, cytological, immunological and biochemical evidence strongly support the hypothesis of

X chromosome conservation and, indeed, up to now there is no single evidence that what is X-linked in one mammalian species is autosomally inherited in another (for review, see also Ohno 1967).

Since the Y chromosome of mammals is believed to have differentiated from one of the homologues of the ancestral pair of sex chromosomes, this suggests that the functional part of the Y should be evolutionarily conserved too, and such evidence also exists (Ohno 1979, Singh et al. 1980, 1981, Singh and Jones 1982, Jones 1983). This, of course, is of vital importance for the discussion which is to follow later, on sex-determining genes located on the sex chromosomes.

In somatic cells of female mammals, one of the two X chromosomes is inactivated, a mechanism thought to compensate for unequal amounts of X chromosomal genes in males and females (Lyon 1961). However, there is evidence that a segment of the distal short arm of the human X, carrying the genes for the Xg blood group system (Race and Sanger 1975), the steroid sulphatase (STS) locus (Mohandas et al. 1979) and a gene (HYB) controlling the expression of a male specific antigen (Wolf et al. 1980a, Wachtel et al. 1980), is never inactivated. Whether this non-inactivated segment is a special feature of the human X chromosome or is a general feature of the ancestral, highly conserved mammalian X chromosome is not known.

An investigation of the number of active STS-gene copies in the X chromosomes of the wood lemming (a detailed description of the wood lemming sex chromosome system appears later in this section) revealed that

STS activity was directly correlated to the number of X chromosomes and unrelated to the phenotypic sex (II). This allowed the conclusion that in the wood lemming the STS gene(s) is also X-linked, but not subject to inactivation.

In the mouse there is also evidence for X-linkage of the STS gene (Gartler and Rivest 1983), but, in contrast, this gene is inactivated (Gartler and Rivest 1983, Crocker and Craig 1983), and there is no other X-linked gene known to escape inactivation in the mouse.

Thus, in man and in the wood lemming, there is a difference in gene dosage between XX individuals on the one hand and XY and XO individuals on the other, with respect to some X-linked genes, while in the mouse evidence for such a difference is lacking. The importance of this will become obvious in the ensuing discussion on sex chromosomal genes involved in sex determination.

Another feature of the effects of X-inactivation was reported on in paper III.

The wood lemming (Myopus schisticolor) is an exceptional mammal since in this species three types of sex chromosomal females occur, namely XX, X*X and X*Y (Fredga et al. 1976). The mutant X* chromosome, cytologically distinguishable from the wild-type X (Herbst et al. 1978), is strongly female determining (or as I prefer, non-male determining) even when present together with a Y chromosome. X*Y females are fertile but, normally, produce daughters only. This is due to a mechanism of mitotic double non-disjunction in the fetal ovary of such females, causing the replacement of the Y chromosome by a copy of the X* in the germ line.

This, then, leads to the production of X*-carrying eggs only (Fredga et al. 1976). However, occasional disturbances of the non-disjunction event result in the relatively frequent occurrence of animals with numerical sex chromosome aberrations such as XO (the X being of paternal origin), X*XY and X*YY (Gropp et al. 1976, Winking et al. 1981). Males are generally of the XY type (Fredga et al. 1976, Herbst et al. 1978).

The X*XY wood lemmings are of special interest since they may occur as phenotypical males (sterile), females (fertile) or hermaphrodites (most likely sterile) (Winking et al. 1981). If one assumes that in these individuals the X or the X* is non-randomly inactivated, the different sexual phenotypes could be explained as follows: Individuals with an inactivated wild-type X in all or in the majority of the cells would develop into females (equivalent to X*Y) while those with an inactive X* would develop into males (equivalent to XY). With random inactivation of the X and X* the animal would become a hermaphrodite.

In paper III, chromosome replication patterns of the X chromosomes of X*XY wood lemmings of male and female phenotypes were studied. (Late replication of an X chromosome indicates inactivation of that chromosome.) It was found that the wild-type X was inactivated in all cells of the female while in the male the X* was the late replicating one. The conclusion, thus, was that non-random X-inactivation, indeed, is responsible for the different sexual phenotypes of wood lemming X*XY individuals. Unfortunately, it has not been possible to investigate any X*XY hermaphrodites.

Only one such animal has been found and cells of that specimen do not exist any more. Based on the findings in the male and the female it was, however, predicted that generally, X*XY hermaphrodites should show mosaic patterns with regard to the type of X chromosome inactivated, a prediction which in the future hopefully can be tested.

SEX DETERMINING GENES

(Papers IV, V, VI, VII and IX)

It may be worthwhile to define what, at the ontogenetic level, is meant by sex determination. The sex of the gonads can not be morphologically distinguished up to a certain stage of development (the time when this can be done depends on the species). The gonadal anlage is said to be indifferent. Primary sex determination, as used in this text, is equivalent to gonadal differentiation, i.e. those events which establish the male or female gonad. By sex differentiation is meant those later events taking place after sex determination and establishing the phenotypic sex. Having defined sex determination, we can now turn to sex determining genes.

Although in all sexual species sex determining genes must exist, these need not necessarily be located in the sex chromosomes. It seems that in mammals (and probably birds) where differentiated sex chromosomes occur, these chromosomes have acquired regulatory roles in sex determination and that the structural genes for sex determination are autosomal. There is now abundant evidence in mammals

for the existence of both types of sex determining genes. This evidence will soon be discussed.

There is at present no adequate general theory of development, at least in the same sense that there are adequate theories of evolution and genetics. The biological complexity of primary processes involved in development - cell division, migration, cell recognition and adhesion, differentiation and cell death - defies simple analysis in terms of lists of genes or gene products and their linear interaction.

It has been proposed that cell - cell recognition occurs by means of action of cell adhesion molecules (CAMs), and several CAMs have now been found in a number of vertebrates (Edelman 1983).

One molecule with the characteristics of a CAM which seems to play an important role in gonadal differentiation is the H-Y antigen.

H-Y ANTIGEN AND SEX DETERMINATION

The H-Y antigen was discovered by Eichwald and Silmsker (1955). These authors reported that when skin grafts from certain strains of inbred mice were exchanged in all possible donor/recipient combinations (i.e., male to female, male to male, female to female and female to male), only male grafts on syngeneic females were rejected. Since the only genetic difference between these males and females was the presence of the Y chromosome in the males the antigen was later named H-Y (histocompatibility Y) antigen (Billingham and Silvers 1960).

It soon became evident that the consistent rejection of male grafts by females, with a median survival time (MST) of approximately 25 days, occurs only in certain inbred strains such as the C57BL/6 (B6) or C57BL/10 (B10). Females of various other strains such as C3H and CBA permanently accept male skin grafts. It was therefore lively debated whether only males of certain inbred strains carry the H-Y antigen. Eventually, the cause of these strain differences was identified. It was demonstrated that the H-2 haplotype of the MHC (major histocompatibility complex) gene complex of the mouse determines the manner of female response to male grafts. Strong rejector strains such as B6 and B10 are H-2^b whereas non-rejectors such as C3H and CBA carry the H-2^k haplotype. H-2^a and H-2^d strains represent weak inconsistent rejectors. With the use of H-2-F₁ hybrid females it could finally be demonstrated that all male mice carry the H-Y antigen (for review, see Simpson 1982).

Attempts to localize the structural gene(s) for H-Y in the mouse have been made, and indicate (Krállová and Démant 1976) or imply (Washburn and Eicher 1983, Kiel-Metzger and Erickson 1984) a location within the T/t-H-2 complex of chromosome 17.

So far, I have referred to H-Y antigen as defined by skin grafting (histogeneically defined).

The first attempts to develop a serological assay for the detection of H-Y were made by Hauschka et al. (1959). Later, Goldberg et al. (1971), Scheid et al. (1972) and Fellous et al. (1978) devised more or less improved cytotoxicity tests directed against different types of male

target cells in vitro. H-Y antisera, raised by grafting of syngeneic male skin or by injection of syngeneic male cells in females of inbred mice or rats, were absorbed with male or female test cells. The residual cytotoxicity of the absorbed antisera was then tested against a male target cell type in the presence of complement. These cytotoxicity tests were a major advance in H-Y research since they made it possible to demonstrate presence or absence of the antigen even on cells from non-inbred species.

It was soon realized that, as a general rule, H-Y was present on male but not on female cells of all mammals tested, thus, indicating evolutionary conservation. In addition, except for erythrocytes (Sachs and Heller 1958) and premeiotic germ cells (Zenzes et al. 1978, Koo et al. 1979), H-Y was shown to be present on all male tissues tested (reviewed by Wachtel 1983).

When it was found that cells of female, but not male, birds carried the H-Y antigen (Wachtel et al. 1975a), the concept of male specificity was changed to heterogametic sex specificity. The designation H-Y has, however, been maintained although some investigators prefer to refer to it as H-W in species with female heterogamety. (This seems to be an unnecessary complication since, as mentioned above and as will be discussed later, the structural gene(s) for H-Y, and thus the antigen itself, does not seem to be coded for by the heteromorphic sex chromosomes.)

THE BIOLOGICAL ROLE OF H-Y ANTIGEN

In 1975, the accumulated data, especially those on the general occurrence of H-Y in the heterogametic sex and its evolutionary conservation, prompted Ohno (Wachtel 1983) to put forward a hypothesis on the biological function of this antigen (Wachtel et al. 1975b).

Ohno suggested that H-Y antigen was the product of mammalian testis-determining genes and, in species with female heterogamety, the corresponding product of ovary-determining genes. The main basis for his hypothesis was that "... widespread evolutionary conservation of a particular cell surface component indicates the invariant persistence of a specific function ..." and "... in the case of H-Y antigen, this invariant specific function may be to direct the indifferent embryonic gonad to develop towards whichever mature gonad, testis or ovary ..." depending on the heterogametic sex of the species in question (Wachtel et al. 1975b).

Critical tests of the hypothesis of H-Y antigen as the primary sex determiner, so far, have not been able to falsify it. Experimental evidence for the verification of the hypothesis has been reviewed by Ohno (1979), Wachtel (1983) and Wolf (1984). Thus, this evidence does not need to be discussed further here.

One piece of evidence, important for the concept of H-Y antigen acting as a CAM-like cell surface molecule is, however, to be mentioned.

As already discussed, H-Y antigen is ubiquitously expressed in the mammalian male. The current concept of the role of H-Y as a testis-organizer is that it is involved in the

formation of seminiferous tubules. To be able to act as a CAM-like molecule a counter molecule (receptor) is required. There is abundant evidence for an H-Y antigen receptor (Ohno 1976, Müller et al. 1978, Müller et al. 1979) and, interestingly, this receptor is gonad-specific but not sex-specific.

Thus, in the male, H-Y antigen can interact with its gonad-specific receptor to initiate the organization of testes, while in the female the absence of H-Y antigen, irrespective of the presence of the receptor, prevents such an organization.

It follows that mammalian individuals with testes should always type H-Y antigen positive ($H-Y^+$), while those with ovaries should type $H-Y^-$ (for species with female heterogamety the reverse applies).

However, the situation is not that straightforward since there is evidence that the presence of H-Y does not necessarily interfere with female development. One explanation for this is that a certain amount of H-Y seems to be a prerequisite for normal development of testes (a threshold effect) (Wolf et al. 1980b).

A biological model which well illustrates this threshold effect on the cellular level is XX/XY chimaeric mice, in which the proportion of XY cells determines whether or not an individual will develop into a male (McLaren 1976, 1978, 1983). This is compatible with the H-Y antigen hypothesis, i.e. the ratio of XY to XX cells in the gonadal anlage determines the amount of H-Y antigen produced and, consequently, the fate of the indifferent gonad.

Thus, the presence of reduced amounts of H-Y antigen in an

individual of female phenotype is not incompatible with the H-Y hypothesis and examples of this are human XO females. Such individuals carry reduced amounts of H-Y (Wolf et al. 1980b and paper IX) and develop into sterile females.

Another very interesting situation, strongly supporting the H-Y hypothesis, is the one concerning XY sex-inversed mice carrying a foreign Y chromosome (Eicher et al. 1982, Gropp 1982). Such mice may occur as females ($H-Y^-$ or with strongly reduced amounts of H-Y), as males ($H-Y^+$) or as $H-Y^+$ hermaphrodites (most frequently with reduced amounts of H-Y) (Wolf in press).

What then about the reverse situation; i.e., have mammalian individuals with testes been found to type $H-Y^-$? There is, indeed, such an individual described. A "male" (the gonads were essentially Leydig cell tumours) XO mouse was reported to type $H-Y^-$ as defined histogeneically but serologically $H-Y^+$ (Melvold et al. 1977). However, as pointed out in papers VII and IX, since that experiment lacked the fundamental scientific requirement of reproducibility, it ought not to be used as evidence for or against H-Y as a testis-organizer.

The paper by Melvold et al. (1977) also raised the question whether different H-Ys are detected with serological and histogeneical methods, and the idea that two H-Y antigens may exist was put forward (Silvers et al. 1982, Simpson et al. 1982). Later evidence (papers VI, VII and IX), however, strongly supports the existence of a single H-Y only.

The homology between H-Y as defined by serology and histogeny will be discussed in the last section of this thesis,

in connection with paper VIII which deals with the methodological difficulties of H-Y serology.

GENETIC REGULATION OF H-Y ANTIGEN EXPRESSION

(Papers IV, V, VI, VII and IX)

Recall the remarkable situation with respect to sex chromosomes in the wood lemming. The fact that X*Y individuals develop into females indicates that the rearranged part of the X* chromosome has lost genetic material important for male sexual development. There is no reason to believe that the Y chromosome should be responsible for the phenomenon of sex inversion since all X*Y females receive their Y chromosomes from normal fertile males.

The wood lemming, thus, has turned out to be a most valuable model species for the genetics of primary sex determination, especially with respect to the X-linked genes involved.

At an early stage of H-Y research (before the knowledge of the gonad-specific receptor) it was felt that H-Y⁺ females would threaten the H-Y hypothesis. It was therefore of particular interest to learn whether X*Y females were H-Y⁺ or H-Y⁻. Wood lemmings of different sex chromosome constitutions were tested for H-Y in the sperm cytotoxicity test developed by Goldberg et al. (1971), and only males were found to type H-Y⁺ (Wachtel et al. 1976). The authors interpreted their results as indication that there was an X-linked "sex-reversal" mutation that blocks the expression of H-Y. These results, thus, fitted the H-Y hypothesis.

With the advance of H-Y research, however, it became clear that the sperm cytotoxicity test was very difficult to evaluate due to extreme reactivity of sperm with naturally occurring heterophil antibodies of the antisera, and difficulties in scoring live and dead spermatozoa (Goodfellow and Andrews 1982, and Fig. 12-1 in Wachtel 1983).

The wood lemming was therefore serologically re-investigated in 1982, using the Raji cell cytotoxicity assay of Fellous et al. (1978), (paper V). It was found that only XX females were H-Y negative. The conclusion was that a deletion, involving both the H-Y repressor and the receptor genes, had occurred in the rearranged short arm of X*. Although H-Y antigen positive, lack of the receptor explained the female sex of X*Y individuals.

The conflicting results by Wachtel et al. (1976) and those just mentioned (V) threw some doubt on H-Y serological methodology (Goodfellow and Andrews 1982). The finding of complete agreement between H-Y serology (V) and histogeny (paper VI) finally settled the wood lemming case. These studies established that wood lemming X*Y females are H-Y antigen positive and, furthermore, led to the conclusion that of the serological assays used in the two wood lemming investigations, the Raji cell assay was the more reliable one (Table 1 of this thesis).

The picture of how the expression of H-Y antigen is genetically regulated emerged from the wood lemming studies, and a model, which in essence was an extension of the one originally proposed by Wolf (1980), could be put forward. Experimental data in support of the model are summarized in Table 2 of this thesis.

Briefly, the structural gene(s) for H-Y (HY) is autosomal. An X-linked gene (HYB) suppresses HY in a dosage dependent manner and is subject to non-inactivation. Double dosage of HYB prevents H-Y expression as in the XX female. Another X-linked gene (HYC), coding for the H-Y gonad-specific receptor, does not escape X-inactivation. By (a) deletion mutation(s), HYB and HYC have been lost by the X* chromosome. Finally, a gene on the Y chromosome (HYA) has an antagonistic effect on HYB (Fig. 1 of this thesis).

Thus, XX wood lemmings are H-Y⁻ due to double dosage of HYB; XY individuals are H-Y⁺ due to the antagonistic effect of HYA on HYB and, due to expression of HYC, develop into males. XO individuals, due to a single dose of HYB, carry reduced amounts of H-Y antigen, insufficient for the development of testes. (Recall the earlier discussion on XX/XY chimaeric mice, XO human females and XY sex inversed mice.)

So far, the proposed model fits the situation in man too.

In wood lemmings carrying the X* chromosome, the situation is quite different. For X*X females (with respect to X-linked genes of the H-Y system, equivalent to XO females) the same applies as for XO wood lemmings. X*Y individuals are H-Y⁺ due to loss of the HYB gene (full H-Y expression) but, since the HVC (receptor) gene, in addition, is lost, they develop into females. For X*O, finally, the same applies as for X*Y females; the additional lack of the Y chromosome is irrelevant (Fig. 1 of this thesis).

For XO female mice there are conflicting data on the H-Y status. There are several reports that these females type histogeneically H-Y⁻ (Celada and Welshons 1963, Haughton et al. 1979, Simpson 1982, Simpson et al. 1982), and one report that such mice type H-Y⁺ (Koo et al. 1983).

Originally, when serologically tested, female XO mice were found to type H-Y⁺ (Engel et al. 1981), but later investigations indicated that gonadal tissue (Engel, personal communication and paper IX) and a variety of somatic tissues of a large number of XO mice typed H-Y⁻ (paper IX).

The reason for these inconsistencies is most likely to be explained by methodological difficulties similar to those reported by Cleve (1981), those of the wood lemming case discussed above, and as generally discussed by Goodfellow and Andrews (1982), and in paper VIII.

Returning to the H-Y status of female XO mice; at present it seems clear that these mice are H-Y⁻ irrespective of the assay used. This means that, while human and wood lemming XO individuals carry the antigen (reduced amounts), XO mice do not. Clearly, there are interspecific differences with respect to **the** regulation of H-Y antigen expression.

These differences are best explained by recalling the available evidence already discussed i.e., that one of the X chromosomes in the mouse is inactivated in toto, while in man and wood lemming part of the X chromosome remains active. Since the HYB gene is X-linked, and in man has been mapped to the region subject to non-inactivation, there is a difference in gene dosage of HYB in human XX and XO individuals. This, most likely, applies to the wood lemming as well (as indicated by the evidence in II), in contrast to the mouse where in XX and XO individuals the same number of HYB genes are expressed (IX).

Before turning to the next and last section, the results of paper IV shall be briefly commented upon.

Male and female European eels were tested, and in this species females were found to carry the H-Y antigen. The implication of these findings, with respect to the question of male or female heterogamety in the eel was discussed, a discussion which in paper I was further extended to include the problems of the genetic regulation of H-Y in lower vertebrates.

HOMOLOGY OF H-Y ANTIGEN AS DEFINED BY DIFFERENT METHODS

(Papers V, VI, VII and IX)

Some discrepancies of H-Y typing - a serologically H-Y positive but histogeneically negative "male" mouse and H-Y negative and positive XO female mice, discussed above - led to speculations about the number of existing H-Y antigens and the proposal that different H-Ys are detected by serology and histogeny (Silvers et al. 1982, Simpson et al. 1982).

Methodological difficulties, responsible for the discordant findings of the H-Y status in XO mice, were pointed out and discussed in the preceeding section and in paper VIII.

The results obtained in papers VI and IX have been particularly informative as to the question of the number of existing H-Y antigens.

Paper VI established that in the wood lemming there is a complete agreement between H-Y serology and histogeny (Table 1 of this thesis).

When it was found that XO mice were serologically H-Y⁻ (IX), the earlier discrepancies of H-Y typing in this species could be eliminated too.

In paper VII, finally, homology of the serological and histogeneical H-Y antigens of man was demonstrated.

Taken together, these results indicate that there is no longer any reason to postulate the existence of more than a single H-Y antigen. Furthermore, these papers were also informative with respect to another problem of H-Y research, i.e., the question whether XX females are serologically

completely H-Y negative or carry residual amounts of the antigen, a question which has confused some authors (Zenzes and Reed 1984).

As to this question the following can be said. It is experimentally evident that as few as 2,500 (Harnasch 1979) or 60,000 (Billingham et al. 1965) XY cells are capable of sensitizing (VI and VII) B6 female mice against H-Y of male skin grafts, and that rejection of male grafts by such sensitized females (expressed as MSTs) is directly proportional to the number of sensitizing XY cells.

In contrast, as many as 20 million XX cells (the largest number tested according to the literature) are unable to influence MSTs of male grafts, showing that XX cells lack the H-Y antigen. Accepting that H-Y as serologically defined, is one and the same as the histogeneically defined antigen (which now seems proven; V, VI, VII and IX), it follows that XX cells are serologically negative too.

The papers of this thesis have, furthermore, demonstrated that the use of the histogeneical and serological H-Y assays in parallel will allow for more precise statements as to the H-Y status of a subject under investigation. With the former system it can be determined whether a given cell type expresses H-Y at all, while the latter system allows for quantification of H-Y.

Exciting subjects for further studies are XY female mice with Y chromosomes of different origins, XX sex reversed mice and different types of sex chromosomal abnormalities in man.

ADDENDUM

During the final preparation of this thesis, McLaren et al. (Nature 312, 552-555) published a paper entitled: "Male sexual differentiation in mice lacking H-Y antigen". For details (partly essential for the discussion below) of this investigation, I refer to the original article.

The male sexual phenotype of XX sex reversed mice is due to a translocation of a fragment (Sxr) from the Y chromosome to the paternal X chromosome. The sex chromosome constitution of these mice is designated X/X^{Sxr} . Since these mice develop as males, the Sxr region is presumed to include testis-determining factors; (H-Y) and, indeed, all X/X^{Sxr} mice so far tested typed H-Y⁺.

McLaren and her colleagues have now found a variant (mutated) form of Sxr, Sxr', which in $X/X^{\text{Sxr'}}$ phenotypically normal, but sterile, males has retained its testis-determining property but has lost its antigenicity. Clearly, $X/X^{\text{Sxr'}}$ mice have testis but type H-Y⁻.

The authors conclude that:

"It seems, therefore, that we are dealing with a variant form of the Sxr region, which retains the testis-determining property but has lost the sequences controlling the expression of H-Y transplantation antigen. We propose that this variant be designated Sxr', with the constitution Tdy⁺ [testis-determining Y-linked gene], Hya⁻, where Hya is the locus (structural or regulatory) controlling the expression of H-Y antigen. Tdy and Hya may be the same locus, or two separate loci.

Our data do not allow us to determine whether Sxr' lacks the Hya locus altogether, or retains it in a modified form such that the antigenicity by which the molecule is defined has been lost."

What seems clear from these experiments is that the part

of H-Y antigen which defines its antigenicity is not necessary (at least in X/X^{Sxr'} mice) for the development of testis.

Interesting in this context is that evidence exists for a human H-Y antigen which has lost its H-Y receptor binding (functional) specificity, but retained its antigenicity (Nagai, Y. et al. In Testicular Development, Structure and Function (Eds. E. Steinberger and A. Steinberger), Raven Press, New York, 1980, pp. 41-47).

The reverse, then, could well apply to the case discussed here.

EPILOGUE

"Whenever possible, I have attempted a synthesis of opposing viewpoints (unless one of them is clearly in error). Where the situation is quite unresolved, I have described the opposing viewpoints in categorical, sometimes almost one-sided, terms in order to provoke a rejoinder, if such is justified. Because I hate beating around the bush, I have sometimes been called dogmatic. I think this is the wrong epithet for my attitude. A dogmatic person insists on being right, regardless of opposing evidence. This has never been my attitude and, indeed, I pride myself on having changed my mind on frequent occasions. However, it is true that my tactic is to make sweeping categorical statements. Whether or not this is a fault, in the free world of interchange of scientific ideas, is debatable. My own feeling is that it leads more quickly to the ultimate solution of scientific problems than a cautious sitting on the fence."

Ernst Mayr

(In: Mayr, E. The Growth of Biological Thought. The Belknap Press of Harvard University Press, Cambridge, 1982)

GENERAL SUMMARY

- I. A large number of histologically sexed European eels were cytogenetically investigated. The investigation revealed that a heteromorphic chromosome pair frequently occurred. With the use of chromosome banding techniques it could be demonstrated that this heteromorphism was due to quantitative differences of constitutive heterochromatin and nucleolar organizing regions and that it was not correlated with sex. Therefore, despite the claim by earlier investigators, it was concluded that in this species heteromorphic sex chromosomes do not exist. Furthermore, a hypothesis that sex determination in the European eel is metagamic, influenced by temperature, was put forward.
- II. The number of active gene copies of the STS gene in the X chromosomes of the wood lemming was investigated. It was found that STS activity was directly correlated to the number of X chromosomes but unrelated to the phenotypic sex. It was therefore suggested that in this species, as in man, the STS gene is X-linked and not subject to X chromosome inactivation.
- III. Replication patterns of the X chromosomes of phenotypical male and female X^*XY wood lemmings were studied. The wild type X was found to be late replicating (X-inactivated) in all cells of the X^*XY female whereas in the X^*XY male the mutated X (X^*) was inactivated in all cells. It was concluded that the phenotypic sex in these animals depends on the type of active X chromosome.

- IV. Anti H-Y antiserum was absorbed with gonadal cells from European eels of both sexes. The antiserum was found to cross-react with female, but not male, gonadal cells. It was therefore suggested that in this species, the female is the heterogametic sex.
- V. Wood lemmings (Myopus schisticolor) were serologically investigated for expression of H-Y antigen. X*Y and X*O females expressed amounts of antigen in the male control range while X*X and XO females expressed the antigen intermediary to fully positive males and negative females. Full expression of H-Y antigen in the absence of testis differentiation was explained by the assumption of a deficiency of the gonad-specific receptor for H-Y antigen, coded for by a gene on the X chromosome but lost by the X* chromosome.
- VI. Groups of C57BL/6 (B6) female mice were sensitized with wood lemming fibroblasts of different sex chromosomal types (XX, XY, X*Y and X*O) and subsequently transplanted with syngeneic male or female skin. Cells of the XY, X*Y and X*O, but not XX, types, sensitized B6 females against syngeneic male, but not female, **grafts**. This allowed the conclusions i) that the graft reactions were H-Y induced and not wood lemming specific, ii) that X*Y and X*O females, as well as males, carry the H-Y antigen, iii) that, in this species at least, the structural gene(s) for H-Y is not located in the Y chromosome, and iv) that in the wood lemming the serological and histogeneical H-Y antigens are one and the same.

VII. It was shown that B6 female mice, sensitized with human XY and XO, but not XX, fibroblasts, rejected syngeneic male skin grafts more rapidly (second set graft reaction). This demonstrated that the antigenic determinants of H-Y antigen of man and mouse are homologous and that human XO females carry the H-Y antigen. Furthermore, the findings allowed a discussion on the identity of H-Y as defined by different methods and on the chromosomal location of the H-Y structural gene(s).

VIII. The importance of the tissues to be tested, antiserum and complement, the testing procedures, quantitative absorption and the treatment of data in H-Y serological research was critically discussed. It was summarized that only with thorough knowledge of the different parameters influencing H-Y serological tests is it possible to perform and interpret them correctly. A further conclusion was that mammalian XX females are completely H-Y negative and that in XY males, although possible, no evidence for inter-individual variation of the amount of H-Y antigen exists.

IX. XX, XY and XO mice were serologically investigated for the expression of H-Y antigen. Organ homogenates of XX and XO females were equally unable to absorb H-Y antibodies of H-Y antisera, indicating that such female mice do not carry the H-Y antigen. The findings were discussed with respect to species specific differences in the regulation of H-Y antigen expression and with attention to the question of the existence of one or several H-Y antigens.

ACKNOWLEDGMENT

Without hesitation, the one man who deserves my most sincere thanks is my teacher Professor Karl Fredga. Apart from having introduced me to the science of genetics he has, through the years, supported me and my research. Equally important; he taught me scientific ethics, how to apply for grants and to play better table-tennis. I do not thank him for his personal friendship, instead I enjoy it.

Professor Ulrich Wolf is thanked for providing laboratory facilities at the Institut für Humangenetik und Anthropologie, Freiburg i. Br., and for his continuous interest in, and support of, my work. His experience as an expert in my field of scientific research is, likewise, highly appreciated.

Professor Arne Lundqvist is thanked for his continuous support and for extremely valuable help during all stages of the preparation of my dissertation.

I also wish to express my gratitude to

- Drs. Nils Mandahl and Bengt Widegren for discussions and personal friendship
- All colleagues and friends at the Institute of Genetics and the Wallenberg Laboratory in Lund and at the Institut für Humangenetik in Freiburg.

The work referred to in this thesis was supported by grants from the Royal Physiographic Society in Lund; the Nilsson-Ehle Foundation, the Erik Philip-Sörensen Foundation, Sven and Lilly Lawski's Foundation for Scientific Research, the Royal Swedish Fishery Board, the Swedish Natural Science

Research Council, the Deutscher Akademischer Austauschdienst,
the Alexander von Humboldt Foundation and the Deutsche
Forschungsgemeinschaft.

REFERENCES

- Allen, G.E. (1978). Thomas Hunt Morgan. The Man and His Science., Princeton University Press, Princeton
- Billingham, R.E. and Silvers, W.K. (1960). Studies on tolerance of the Y chromosome antigen in mice. J. Immunol. 85, 14-26
- Billingham, R.E., Silvers, W.K. and Wilson, D.B. (1965). A second study on the H-Y transplantation antigen in mice. Proc. Roy. Soc. London B. 163, 61-89
- Celada, F. and Welshons, W.J. (1963). An immunogenetic analysis of the male antigen in mice utilizing animals with an exceptional chromosome constitution. Genetics 48, 139-151
- Chen, T.R. and Ebeling, A.W. (1966). Probable male heterogamety in the deep sea fish Bathylagus wesethi (Teleostei: Bathylagidae). Chromosoma 18, 88-96
- Chen, T.R. and Ruddle, F.H. (1970). A chromosome study of four species and a hybrid of the killifish genus Fundulus (Cyprinodontidae). Chromosoma 29, 255-267
- Cleve, H. (1981). H-Y antigen and sex determination. In Protides of the biological fluids Vol. 29, pp 3-12 (Ed. H. Peeters), Pergamon Press, Oxford
- Conover, D.O. and Kynard, B.E. (1981). Environmental sex determination: interaction of temperature and genotype in fish. Science 213, 577-579

- Crocker, M. and Craig, I. (1983). Variation in regulation of steroid sulphotase locus in mammals. Nature 303, 721-722
- Cuénot, L. (1899). Sur la détermination due sexe chez les animaux. Bull. Sci. Fr. Belg. 32, 461-534
- D'Ancona, U. (1960). The life cycle of the Atlantic eel. Symp. zool. Soc. London. 1, 61-75
- Edelman, G.M. (1983). Cell Adhesion Molecules. Science 219, 450-457
- Eicher, E.M., Washburn, L.L., Whitney, J.B. III. and Morrow, K.E. (1982). Mus poschiavinus Y chromosome in the C57BL/6J murine genome causes sex reversal. Science 217, 535-537
- Eichwald, E.J. and Silmsen, C.R. (1955). Untitled communication. Transplant. Bull. 2, 148-149
- Engel, W., Klemme, B. and Ebrecht, A. (1981). Serological evidence for H-Y antigen in XO female mice. Hum. Genet. 57, 68-70
- Fellous, M., Günther, E., Kemler, R., Wiels, J., Berger, R., Guenet, J.L., Jakob, H. and Jacob, F. (1978). Association of the H-Y male antigen with β_2 -microglobulin on human lymphoid and differentiated mouse teratocarcinoma cell lines. J. Exper. Med. 147, 58-70
- Ferguson, M.W. and Joanen, T. (1982). Temperature of egg incubation determines sex in Alligator mississippiensis. Nature 296, 850-853
- Frédéric, J. (1961). Contribution a l'étude du caryotype chez le poulet. Arch. Biol. 72, 185-209
- Fredga, K., Gropp, A., Winking, H. and Frank, F. (1976). Fertile XX- and XY-type females in the wood lemming Myopus schisticolor. Nature 261, 225-227

- Gartler, S.M. and Rivest, M. (1983). Evidence for X-linkage of steroid sulfatase in the mouse: steroid sulfatase levels in oocytes of XX and XO mice. Genetics 103, 137-141
- Goldberg, E.H., Boyse, E.A., Bennett, D., Scheid, M. and Carswell, E.A. (1971). Serological demonstration of H-Y (male) antigen on mouse sperm. Nature 232, 478-480
- Goodfellow, P.N. and Andrews, P.W. (1982). Sexual differentiation and H-Y antigen. Nature 295, 11-13
- Gropp, A. (1982). Sex reversal in XY mice by a foreign Y chromosome. In Prospects for Sexing Mammalian Sperm (Eds. R.P. Amann and G.E. Seidel Jr.), Colorado Assoc. Univ. Press, Boulder
- Gropp, A., Winking, H., Frank, F., Noack, G. and Fredga, K. (1976). Sex chromosome aberrations in wood lemmings (Myopus schisticolor). Cytogenet. Cell Genet. 17, 343-358
- Harnasch, D. (1979). A rapid method for preimmunisation and grafting of mice with ear skin. Transplantation 28, 402-404
- Haughton, G., Daly, J.A. and Wikstrand, C.J. (1979). An investigation of allograft tolerance: Discordant reactivity to murine H-Y incompatible lymphoid cell (PEC) and skin grafts. Transplantation 27, 203-207
- Hauschka, T.S., Grinnell, S.T., Meagher, M. and Amos, D.B. (1959). Sex-linked incompatibility of male skin and primary tumors transplanted to isologous female mice. In Genetics and Cancer, Univ. Texas Press, Austin
- Henking, H. (1891). Untersuchungen über die ersten Entwicklungsvorgänge in den Eiern der Insekten. II. Über Spermatogenese und deren Beziehung zur Entwicklung bei Pyrrhocoris apterus. Z. Wiss. Zool. Abt. A51, 685-736

- Herbst, E.W., Fredga, K., Frank, F., Winking, H. and Gropp, A. (1978). Cytological identification of two X-chromosome types in the wood lemming (Myopus schisticolor). Chromosoma 69, 185-191
- Jones, K.W. (1983). Evolution of sex chromosomes. In Development in Mammals, Vol. 5 (Ed. M.H. Johnson), Elsevier, Amsterdam
- Kiel-Metzger, K. and Erickson, R.P. (1984). Regional localization of sex-specific Bkm-related sequences on proximal chromosome 17 of mice. Nature 310, 579-581
- Koo, G.C., Mittl, L.R. and Goldberg, C.L. (1979). Expression of H-Y antigen during spermatogenesis. Immunogenetics 9, 293-296
- Koo, G.C., Reidy, J.A. and Nagamine, C.M. (1983). H-Y antigen in XO mice. Immunogenetics 18, 37-44
- Králová, J. and Démant, P. (1976). Expression of the H-Y antigen on Thymus Cells and Skin: Differential Genetic Control Linked to K end of H-2. Immunogenetics 3, 583-594
- Lyon, M.F. (1961). Gene action in the X-chromosome of the mouse (Mus musculus, L.). Nature 190, 372-373
- McClung, C.E. (1902). The accessory chromosome - sex determinant? Biol. Bull. 3, 43-84
- McLaren, A. (1976). Mammalian Chimaeras. Developmental and Cell Biology Series (Eds. M. Abercrombie, D. Newth and J.G. Torrey), Cambridge Univ. Press, Cambridge
- McLaren, A. (1978). Sexual Differentiation in Mammalian Chimaeras and Mosaics. In Genetic Mosaics and Cell Differentiation (Ed. W.J. Gehring), Springer-Verlag, Berlin
- McLaren, A. (1983). Sex Reversal in the Mouse. Differentiation 23, S93-S98

- Melvold, R.W., Kohn, H.I., Bergman, G. and Fawcett, D.W. (1977). Evidence suggesting the existence of two H-Y antigens in the mouse. Immunogenetics 5, 33-41
- Mohandas, T., Shapiro, L.J., Sparkes, R.S. and Sparkes, M.C. (1979). Regional assignment of the steroid sulfatase-X-linked ichthyosis locus: Implications for a noninactivated region on the short arm of human X chromosome. Proc. Natl. Acad. Sci. USA 76, 5779-5783
- Morgan, T.H. (1926). The Theory of the Gene., Yale Univ. Press, New Haven, p.242
- Morreale, S.J., Ruiz, G.J., Spotila, J.R. and Standora, E.A. (1982). Temperature-dependent sex determination: current practices threaten conservation of sea turtles. Science 216, 1245-1247
- Müller, U., Aschmoneit, I., Zenzen, M.T. and Wolf, U. (1978). Binding studies of H-Y antigen in rat tissues: Indication for a gonad specific receptor. Hum. Genet. 43, 151-157
- Müller, U., Wolf, U., Siebers, J.W. and Günther, E. (1979). Evidence for a gonad-specific receptor for H-Y antigen: Binding of exogenous H-Y antigen to gonadal cells is independent of β_2 -microglobulin. Cell 17, 331-335
- Ohno, S. (1961). Sex chromosomes and microchromosomes of Gallus domesticus. Chromosoma 11, 484-498
- Ohno, S. (1967). Sex chromosomes and sex-linked genes. Springer-Verlag, Berlin
- Ohno, S. (1974). Protochordata, Cyclostomata and Pisces. In Animal Cytogenetics Vol. 4: Chordata 1 (Ed. B. John), Gebr. Borntraeger, Berlin
- Ohno, S. (1976). Major regulatory genes for mammalian sexual development. Cell 7, 315-321

- Ohno, S. (1979). Major Sex Determining Genes. Springer-Verlag, Berlin
- Race, R. and Sanger, R. (1975). Blood Groups in Man. Blackwell Sci. Publ., Oxford
- Sachs, L. and Heller, E. (1958). The sex-linked histocompatibility antigens. J. Natl. Cancer Inst. 20, 555-561
- Scheid, M., Boyse, E.A., Carswell, E.A. and Old, L.J. (1972). Serologically demonstrable alloantigens of mouse epidermal cells. J. Exper. Med. 135, 938-955
- Schmid, W. (1962). DNA replication patterns of the heterochromosomes in Gallus domesticus. Cytogenetics 1, 344-352
- Silvers, W.K., Gasser, D.L. and Eicher, E.M. (1982). H-Y antigen, serologically detectable male antigen and sex determination. Cell 28, 439-440
- Simpson, E. (1982). The role of H-Y as a minor transplantation antigen. Immunology Today 3, 97-106
- Simpson, E., McLaren, A. and Chandler, P. (1982). Evidence for two male antigens in mice. Immunogenetics 15, 609-614
- Singh, L. and Jones, K.W. (1982). Sex reversal in the mouse (*Mus musculus*) is caused by a recurrent nonreciprocal crossover involving the X and an aberrant Y chromosome. Cell 28, 205-216
- Singh, L., Purdom, I.F. and Jones, K.W. (1980). Sex chromosome associated satellite DNA. Evolution and conservation. Chromosoma 79, 137-157
- Singh, L., Purdom, I.F. and Jones, K.W. (1981). Conserved sex-chromosome-associated nucleotide sequences in eukaryotes. Cold Spring Harb. Symp. Quant. Biol. 45, 805-813

- Sola, L., Camerini, B. and Cataudella, S. (1984). Cyto-genetics of Atlantic eels: C- and G-banding, nucleolus organizer regions, and DNA-content. Cytogenet. Cell Genet. 38, 206-210
- Uyeno, T. and Miller, R.R. (1971). Multiple sex chromosomes in a Mexican cyprinodont fish. Nature 231, 452-453
- Wachtel, S.S. (1983). H-Y antigen and the biology of sex determination. Grune & Stratton, New York
- Wachtel, S.S., Koo, G.C. and Boyse, E.A. (1975a). Evolutionary conservation of H-Y ("male") antigen. Nature 254, 270-272
- Wachtel, S.S., Koo, G.C., Breg, W.R. and Genel, M. (1980). H-Y antigen in X,i(Xq) gonadal dysgenesis: evidence of X-linked genes in testicular differentiation. Hum. Genet. 56, 183-187
- Wachtel, S.S., Koo, G.C., Ohno, S., Gropp, A., Dev, V.G., Tantravahi, R., Miller, D.A. and Miller, O.J. (1976). H-Y antigen and the origin of XY female wood lemming (Myopus schisticolor). Nature 264, 638-639
- Wachtel, S.S., Ohno, S., Koo, G.C. and Boyse, E.A. (1975b). Possible role for H-Y antigen in the primary determination of sex. Nature 257, 235-236
- Washburn, L.L. and Eicher, E.M. (1983). Sex reversal in XY mice caused by dominant mutation on chromosome 17. Nature 303, 338-340
- Wilson, E.B. (1905). Studies on chromosomes. I. The behaviour of the idiochromosomes in Hemiptera. J. Exp. Zool. 2, 371-405

- Winking, H., Gropp, A. and Fredga, K. (1981). Sex Determination and Phenotype in Wood Lemmings with XXY and Related Karyotypic Anomalies. Hum. Genet. 58, 98-104
- Wolf, U. Genes of the H-Y antigen system and their expression in mammals. In Progress and Topics in Cytogenetics. The Cytogenetics of the Mammalian Y Chromosome (Ed. A.A. Sandberg), A.R. Liss Inc., New York (in press)
- Wolf, U. (1980). Studies on H-Y antigen in disorders of sexual development. In Act. Gynécol. (11^e série), Mason, Paris
- Wolf, U. (1984). In Aspects of human genetics (Eds. C. San Roman Cos-Gayon and A. McDermott), Karger, Basel
- Wolf, U., Fraccaro, M., Mayerová, A., Hecht, T., Maraschio, P. and Hameister, H. (1980a). A gene controlling H-Y antigen on the X chromosome. Hum. Genet. 54, 149-154
- Wolf, U., Fraccaro, M., Mayerová, A., Hecht, T., Zuffardi, O. and Hameister, H. (1980b). Turner syndrome patients are H-Y positive. Hum. Genet. 54, 315-318
- Zenzes, M.T., Müller, U., Aschmoneit, I. and Wolf, U. (1978). Studies on H-Y antigen in different cell fractions of the testis during pubescence. Immature germ cells are H-Y antigen negative. Hum. Genet. 45, 297-303
- Zenzes, M.T. and Reed, E.T. (1984). Variability in serologically detected male antigen titer and some resulting problems: A critical review. Hum. Genet. 66, 103-109

Table 1. An overview of published data on the H-Y antigen status (-, +, or $\pm$)[§] in wood lemmings (Myopus schisticolor) of different sex chromosome constitutions.

Assay	Sex chromosome constitution						Reference
	XX	XY	X*X	X*Y	XO	X*O	
Sperm cytotoxicity test	- [†]	+	nt? [†]	-	nt	nt	Wachtel et al. (1976)
Raji cell cytotoxicity test	-	+	$\pm$	+	$\pm$	+	Wiberg et al. (1982) (V)
Transplantation (original definition of H-Y)	-	+	nt	+	nt	+	Wiberg and Günther (1985) (VI)

[§] -, no; +, full; and $\pm$, reduced expression of H-Y antigen

[†] At the time of this investigation the X and X* chromosomes could not yet be cytologically distinguished. X*X animals may therefore be represented in the group of XX animals tested.

nt, not tested

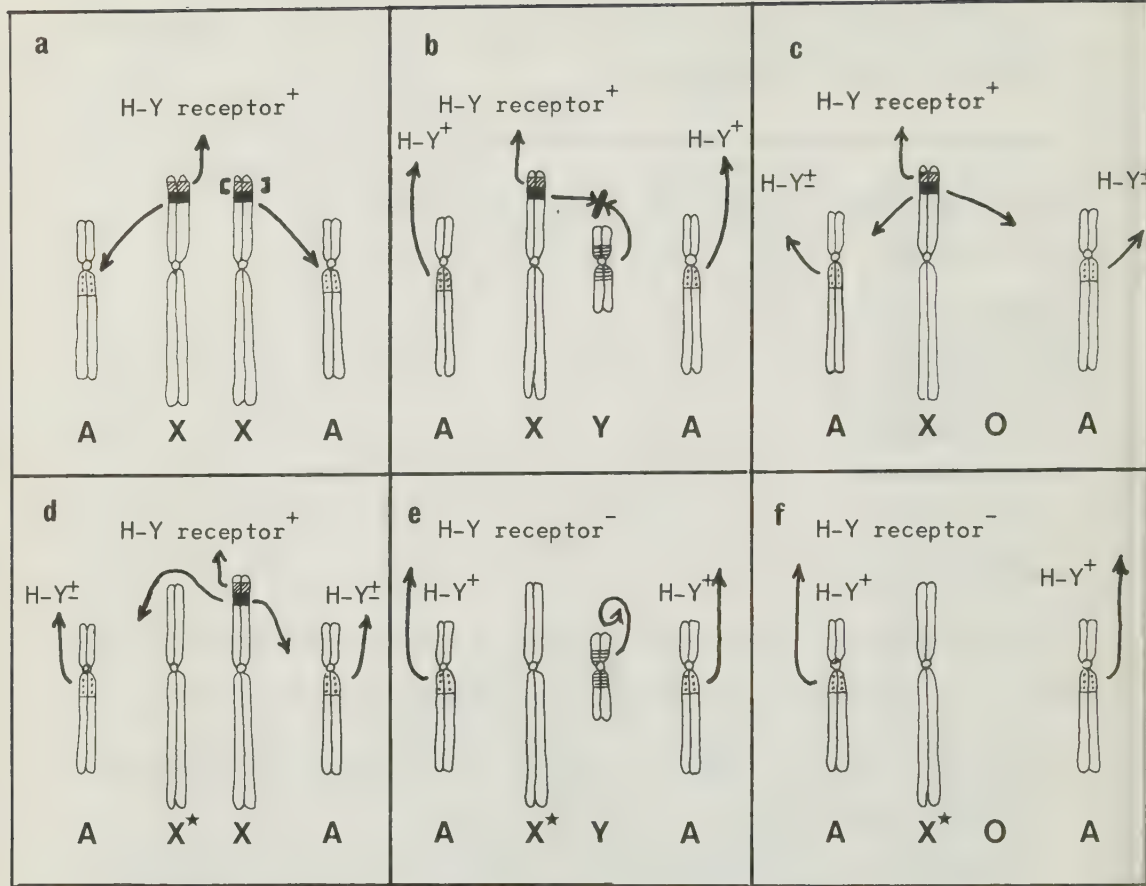
Table 2. A synopsis of the results and conclusions of paper V of this thesis: The expression of genes of the H-Y antigen system in the wood lemming.

Sex chromosomes	XY	XX	X*Y	X*X	XO	X*O
H-Y antigen [§]	+	-	+	+r	+r	+
Number of H-Y receptor genes	1	2	0	1	1	0
Presumptive num- ber of active H-Y receptor genes	1	1	0	1	1	0
Gonad	Te	Ov	Ov	Ov	Ov	Ov
Phenotypic sex	♂	♀	♀	♀	♀	♀

§ -, no; +, full; and +r, reduced expression of H-Y antigen

† the H-Y receptor gene is assumed to be subject to X-inactivation (see Note in the legend to Fig. 1 of this thesis)

|| Te, testis; Ov, ovary. Testis is developed only if the H-Y receptor is present simultaneously with sufficient amount of H-Y antigen (threshold effect)



Symbols:



HY; autosomal H-Y antigen structural gene(s). The autosomes drawn are fictional.



HYB; X-linked repressor gene repressing the HY gene(s). This gene escapes X-inactivation.



HYC; X-linked gene for the gonad-specific H-Y receptor. The location (as in the figure) of this gene is arbitrary. HYC is subject to X-inactivation.



HYA; Y-linked regulatory gene antagonistic to the HYB gene (product).

Figure 1. Model for the genetic regulation of the genes of the H-Y antigen system in the wood lemming.

- (a), XX female: Double dosages of the X-linked HYB repress the autosomal H-Y gene(s) (HY) resulting in H-Y negative females. The H-Y receptor can not exert its functional role in the absence of H-Y antigen.
- (b), XY male: The antagonistic effect of the Y-linked HYA gene on HYB allows for full expression of H-Y antigen, and in the presence of the H-Y receptor testicular development is induced.
- (c), XO female: A single dosage of the HYB gene is insufficient for full suppression of the HY gene(s) and results in expression of reduced amounts of the H-Y antigen which, in turn, are insufficient for the development of testis.
- (d), X*X female: The X* chromosome has lost the HYB and HYC genes and, thus, with respect to the genes of the H-Y antigen system, X*X females are equivalent to XO females.
- (e), X*Y female: The X* chromosome lacks the HYB repressor gene resulting in fully H-Y positive animals. Additional lack of the HYC gene (the H-Y receptor gene) prevents male development. A functional HYA gene is irrelevant for the expression of H-Y antigen in these females.
- (f), X*O female: These females resemble the X*Y females, except for the absence of the Y chromosome, and are fully H-Y antigen positive.

A, autosome; X, wild-type X chromosome; X*, mutated X chromosome; Y, Y chromosome; O, absence of a second sex chromosome; -, no; +, full; and $\pm$, reduced expression respectively.

[] in (a) indicates X-inactivated gene.

Note: Information from the X-inactivation studies of X*XY animals of different sexes (paper III) suggest that the HYC gene is subject to X-inactivation. If this was not the case, the X*XY females (which have an active X*) would develop into males due to expression of the HYC gene of the otherwise inactivated wild-type X chromosome.

Druckerei Johannes Krause, Freiburg und Umkirch
1985